Peer Review
Experimental Toxicology Division
NHEERL

CUMULATIVE RISK

Integrated Disinfection Byproducts Mixtures Research

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ENVIRONMENTAL ISSUE

- Chemical disinfection of water:
  - decreases morbidity and mortality from water-borne disease;
  - results in the formation of disinfection byproducts (DBPs).
- Hundreds (> 500) of DBPs have been identified but ~ half the mass of total organic halide (TOX) formed by chlorination of water has not been identified.
- Human exposure to highly complex mixtures of DBPs is ubiquitous and dependent on factors such as source water characteristics, disinfectants used and distribution system time.
- Epidemiological studies suggest possible associations between human consumption of chlorinated drinking water and reproductive/developmental health effects and cancer.
- Single chemical studies currently available in experimental animals cannot explain the health effects observed in some epidemiological studies.

PROJECT GOAL

- Improved estimation of the potential human health risks associated with exposure to DBP mixtures formed by chemical disinfection of water.

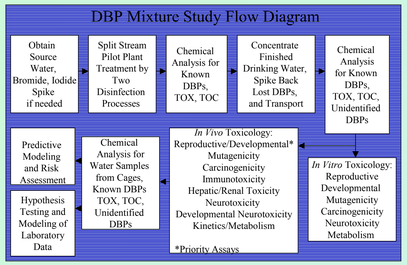
PARTICIPATING LABORATORIES/ORGANIZATIONS

- U.S. Environmental Protection Agency, Office of Research and Development
  - o National Center for Environmental Assessment (NCEA), Cincinnati, OH
  - o National Exposure Research Laboratory (NERL), Athens, GA
  - o National Health and Environmental Effects Research Laboratory (NHEERL), Research Triangle Park, NC
  - o National Risk Management Research Laboratory (NRMRL), Cincinnati, OH
- University of North Carolina (UNC), Chapel Hill, NC
- Metropolitan Water District of Southern California (MWDSC), LaVerne, Ca
- Virginia Commonwealth University (VCU), Richmond, VA
- Syracuse Research Corporation (SRC), Syracuse, NY

EXPERTISE BROUGHT TO THE PROJECT

Water Treatment Technology/Engineering	Toxicology (In Vivo and In Vitro)
Water Concentration	Reproductive
Quantitative Analysis, Commonly Measured DBPs	Developmental
Quantitative Analysis, Nonroutine DBPs	Carcinogenicity
Qualitative Comprehensive Analysis, DBPs	Mutagenicity
Qualitative Comprehensive Analysis, other Chemicals	Pharmacokinetics/Dosimetry
Chemical Mixtures	Metabolism
Statistics	Immunotoxicology
Exposure Assessment	Neurotoxicology
Risk Assessment	Developmental Neurotoxicology
Dose-Response Modeling	Hepatotoxicology
Physiologically-Based Pharmacokinetic Modeling	Renal Toxicology
Relational Data Base Development	Bioassay-Directed Fractionation
	Genomics

DBP Mixture Study Flow Diagram



The flow diagram and the accompanying research concept proposal (Integrated Disinfection ByProducts Mixtures Research: Toxicological and Chemical Evaluation of Alternative Disinfection Scenarios) present a series of studies that are logical to follow, but difficult to implement due to outstanding questions identified by the Research Team:

- Can methods be developed for concentration of large quantities of water such that the DBPs remain in a water matrix?
- Are representative, toxicologically significant DBPs formed during disinfection?
- Does the concentration methodology, that includes spike-back of lost volatile DBPs, result in adequate DBP recovery?
- Are the DBPs stable during transport, storage and time on the animal cages?
- Will the animals drink the concentrated water samples?

•Preliminary experiments (the Trial Run) were conducted to answer these questions.

Concentrating the Sample

Concentrate Production Using Reverse Osmosis Membrane

Reverse Osmosis Concentration - RO
Organics/DBPs are kept in the water phase
Membranes provide a high rate of concentrate production relative to XAD-resin extraction

Spike Back 'Lost' Components - "replace' the predominant known DBPs lost during the concentration procedures
4 THMs, 9 HAAs, 4 HANs, bromate, chlorate

Chemical Analysis

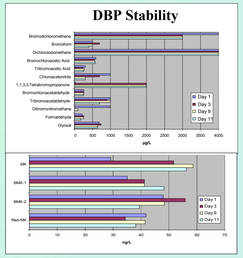
Perform **extensive chemical analyses** at multiple junctures-
Quantitative analyses - **up to 80 DBPs**
Qualitative comprehensive chemical/DBPs identification for all chemicals and DBPs detected by high-resolution GC/MS (gas chromatography/mass spectrometry) analyses

Selected Compounds Identified by GC/MS in Chlorinated Water Concentrated by RO

- **Trihalomethanes:**
  - Bromodichloromethane
  - Dibromochloromethane
  - Bromoform
- **Iodinated halomethanes**
  - Chloroiodomethane
  - Dichloroiodomethane
  - Bromochloroiodomethane
- **Haloacetonitriles**
  - Dibromoacetonitrile
- **Halogenated Furanones**
  - MX
  - BMX-1
  - BMX-2
- **Halonitromethanes**
  - Dichloronitromethane
  - Bromochloronitromethane
- **Haloaldehydes**
  - Bromochloroacetaldehyde
  - Dibromoacetaldehyde
  - Bromodichloroacetaldehyde
  - Trichloroacetaldehyde
  - Iodoacetaldehyde
  - Chlorodibromoacetaldehyde
- **Haloketones**
  - Chloropropanon
  - Dichloropropanone

Qualitative and Quantitative chemical analysis showed that representative DBPs were formed.

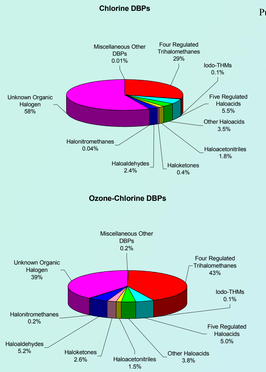
DBP Stability



Stability of representative DBPs in concentrate of ozonated-chlorinated drinking water. Stability data were also available for Day 5 and 7 for some DBPs. However, only Days 1, 3, 9, and 11 are shown here for simplicity. Concentrations for dichloroiodomethane, chloroacetonitrile, 1,1,3,3-tetrabromopropanone, tribromoacetaldehyde, and dibromonitromethane have been multiplied by 100 to enable visual comparisons with other DBPs on the chart.

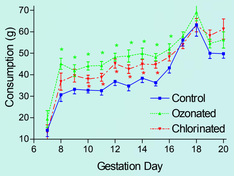
As illustrated here, most DBPs appeared stable during both ideal storage [comparing days 1 and 9] (in the dark at 4°C, in glass/stainless steel/teflon container with no headspace) and while on the animal cages [comparing days 3 and 11].

Separating The TOX (total organic halogen) Formed During Water Disinfection into The Known and Unknown Fractions



Relative amounts of halogenated DBPs as a proportion of the total organic halide (TOX) in the concentrates of (a) chlorinated drinking water and (b) ozonated-chlorinated drinking water.

Will rats drink the DBP concentrates? Yes



Example Trial Run Toxicology Data

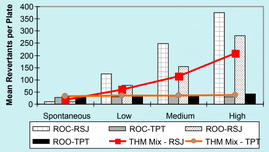
Summary of Developmental Data

Group:	Control	Ozonated	Chlorinated
No. Dams	19	20	20
Delivered GD 21	5	12	8
Delivered GD 22	14	8*	12
With Live Pups	19	20	20
No. Implantation Sites	15.0 ± 0.4	15.0 ± 0.2	15.1 ± 0.2
Live Pups Day 1	14.0 ± 0.4	14.1 ± 0.4	13.7 ± 0.4
Live Pups Day 6	13.9 ± 0.4	14.0 ± 0.4	13.6 ± 0.4
Percent LossPrenatal	6.5 ± 2.2	5.8 ± 2.0	9.2 ± 2.4
Postnatal	0.8 ± 0.5	1.3 ± 1.0	0.4 ± 0.4
Pup Weight (g)Day 1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Day 6	13.1 ± 0.3	13.2 ± 0.3	12.8 ± 0.2

*Gestation lengths were significantly shorter ($p<0.05$) than in the other groups.

No adverse effects were seen in a short-term *in vivo* developmental toxicity screen (Chernoff/Kavlock assay) at ~125-130-fold concentration.

Salmonella mutagenicity of volatile components of the water concentrates



Salmonella mutagenicity of the volatile components of the disinfectant byproduct water samples using tester strains TA1535-RSJ with rat GSH S-transferase T1-1 (+GST), and the -GST TA1535-TPT strain. Samples for this assay were concentrated using reverse osmosis and were either ozonated, ROO; chlorinated, ROC; or were an artificial mixture of THMs, THM mix. All samples were tested without an exogenous activation system (S9).

Mutagenicity due to the volatile components was detected by use of *Salmonella* strains transfected with glutathione S-transferase. A mixture of the 4 regulated THMs assayed at their concentrations in the water concentrates accounted for only part of the observed mutagenicity.

CURRENT ACTIVITIES

- Conduct Power calculations.
- Revise the proposed experimental plan for the full study based on.
  - The information and lessons learned from the pretrial and trial runs and on-going research to refine concentration methodology.
  - The comments of the external expert peer-review panel and internal Agency experts.
  - Power calculation results and mixtures experimental design considerations.
- Develop QA/QC plan and set up data base for the full study using NuGenesis software.

PROGRESS TO DATE

- Developed a detailed research proposal for the full study.
- Extensive internal review of the research proposal by Agency experts.
- External peer-review of the research proposal by a panel of experts.
- Conducted trial run to provide answers to questions that impact the design and conduct of the full study.

ANTICIPATED USES OF DATA FOR RISK ASSESSMENT AND FUTURE RESEARCH

- Use toxicology results from multi-generation reproductive study to address the existence of reproductive risks in humans exposed to complex DBP mixtures.
- Use toxicology results from aberrant crypt assays to address the existence of a mechanism of action for bladder cancer in humans exposed to complex DBP mixtures.
- Provide an improved chemical characterization of the routinely unidentified DBPs.
- Estimate and compare the chemical composition and health risks across the two disinfection types.
- Use exposure data base, characterized by multiple chemical analyses performed on the same sample over time, to develop and refine new exposure models.
- Develop and refine new risk assessment methods for complex mixtures.

IMPACT

- This research is directly responsive to the mandate of the Safe Drinking Water Act Amendments of 1996 requiring EPA to develop new approaches to the study of mixtures found in drinking water.
- New methodology has been developed for concentration of DBPs from disinfected water in a matrix suitable for *in vivo* toxicological investigation.

FUTURE DIRECTIONS

- Conduct the full study to gather extensive toxicological data combined with comprehensive chemical characteristics.
- Use the methods/techniques/procedures developed to conduct joint chemical and toxicological characterization of multiple drinking water disinfection treatment t schemes across a variety of source waters of differing characteristics.

SOLVING AGENCY PROBLEMS